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Assessing Important Horseshoe Crab Spawning Areas and Examination of Spring Red Knot Diet in Coastal Georgia Using DNA Barcoding

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The Center for Conservation Biology is an organization dedicated to discovering innovative solutions to environmental problems that are both scientifically sound and practical within today's social context. Our philosophy has been to use a general systems approach to locate critical information needs and to plot a deliberate course of action to reach what we believe are essential information endpoints.

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EXECUTIVE SUMMARY

The *rufa* subspecies of the Red Knot (*Calidris canutus*) has declined significantly in the past 35 years, leading to federal listing (US Fish and Wildlife Service Federal Register Vol. 79 No. 238, 2014a) under the Endangered Species Act in the United States (16 U.S.C. 1531 *et. seq*) and Canada (COSEWIC 2007, SARA 2007). The determination of regional population estimates and identification of major stopover sites are considered to be the highest priority for the Georgia Department of Natural Resources State Wildlife Action Plan (2015), the Atlantic Flyway Shorebird Business Strategy (Winn et al. 2013), the US Shorebird Plan (Brown et al. 2001), the USFWS Red Knot Spotlight Species Action Plan (2010), and the Western Hemisphere Shorebird Reserve Network (WHSRN) Red Knot Conservation Plan for the Western Hemisphere (Niles et al. 2010a). The Georgia Department of Natural Resources State Wildlife Action Plan ranks the Red Knot as a high priority species (with state status of "Rare") and ranks research of the Red Knot as one of the primary conservation actions needed within the state. The rufa population undertakes extreme migrations with annual movement distances that range up to 30,000 km (Harrington 2001, Niles et al. 2010b). One component of their complex annual cycle is a final refueling event at terminal staging areas in May commencing with a long-distance non-stop flight to breeding grounds in the high Arctic (Morrison and Harrington 1992, Harrington and Flowers 1996, Niles et al. 2008, Niles et al. 2012, SC DNR unpublished data).

Conditions within spring staging sites along the western Atlantic Coast are critical to the future of the rufa population. Birds must build a large enough energy reserve to complete their flight to the Arctic and arrive with enough surplus to initiate reproduction and take advantage of the short breeding season (Morrison and Hobson 2004, Morrison et al. 2007). Although considerable work has been conducted to identify prey resources used by Red Knots for refueling within the mid-Atlantic region (Tsipoura and Burger. 1999, Truitt et al. 2001, Cohen et al. 2010), very little is known about prey use within southern staging sites (Takahashi 2016). Horseshoe Crab eggs (HSC) provide a critical easily digestible caloric resource during spring migration along the Atlantic coast (Gillings et al. 2007, Haramis et al. 2007).

Center for Conservation Biology staff delineated 19 HSC spawning sites including 11 high use sites on the Georgia Coast. Land managers, biologists, and other local experts assisted with initial discovery of most spawning sites. We collected 49 Red Knot fecal samples from Tybee Bar and 30 from Little Egg Island between April and May of 2018. Of these DNA samples, 20 successfully generated PCR products using the universal primers and were sequenced. One Red Knot fecal sample (TB.F.33) showed *Donax variabilis* (clam) sequences only, though visually all samples collected from Tybee Bar were dominated by crushed *Donax* shells. Two Red Knot (LEI.F.27 and LEI.F.29) samples showed *Limulus polyphemus* (HSC) sequences only, though visually all 30 samples collected on Little Egg Island were comprised almost entirely of partially digested HSC eggs.

BACKGROUND

Context

The *rufa* subspecies of the Red Knot (*Calidris canutus*) has declined significantly in the past 35 years, leading to federal listing (US Fish and Wildlife Service Federal Register Vol. 79 No. 238, 2014a) under the Endangered Species Act in the United States (16 U.S.C. 1531 *et. seq*) and Canada (COSEWIC 2007, SARA 2007). The determination of regional population estimates and identification of major stopover sites are considered to be the highest priority for the Georgia Department of Natural Resources State Wildlife Action Plan (2015), the Atlantic Flyway Shorebird Business Strategy (Winn et al. 2013), the US Shorebird Plan (Brown et al. 2001), the USFWS Red Knot Spotlight Species Action Plan (2010), and the Western Hemisphere Shorebird Reserve Network (WHSRN) Red Knot Conservation Plan for the Western Hemisphere (Niles et al. 2010a). The Georgia Department of Natural Resources State Wildlife Action Plan ranks the Red Knot as a high priority species (with state status of "Rare") and ranks research of the Red Knot as one of the primary conservation actions needed within the state. The rufa population undertakes extreme migrations with annual movement distances that range up to 30,000 km (Harrington 2001, Niles et al. 2010b). One component of their complex annual cycle is a final refueling event at terminal staging areas in May commencing with a long-distance non-stop flight to breeding grounds in the high Arctic (Morrison and Harrington 1992, Harrington and Flowers 1996, Niles et al. 2008, Niles et al. 2012, SC DNR unpublished data).

Conditions within spring staging sites along the western Atlantic Coast are critical to the future of the rufa population. Birds must build a large enough energy reserve to complete their flight to the Arctic and arrive with enough surplus to initiate reproduction and take advantage of the short breeding season (Morrison and Hobson 2004, Morrison et al. 2007). Although considerable work has been conducted to identify prey resources used by Red Knots for refueling within the mid-Atlantic region (Tsipoura and Burger. 1999, Truitt et al. 2001, Cohen et al. 2010), very little is known about prey use within southern staging sites (Takahashi 2016). Horseshoe Crab eggs (HSC) provide a critical easily digestible caloric resource during spring migration along the Atlantic coast (Gillings et al. 2007, Haramis et al. 2007).

Due to the small size of their prey, mode of foraging and rapid feeding behavior, quantifying prey use by red knots has been extremely challenging. Except for older studies that have used gut contents, virtually all studies have inferred diet from knot distribution and associated prey sampling within used habitats. Development of a more direct approach to diet assessment would lead to a better understanding of the spatial and temporal dynamics of prey use that would greatly benefit management decisions. If effective, DNA barcoding of prey within feces represents both a more direct, more efficient and less expensive approach to diet assessment.

ACTIVITIES and OBJECTIVES

Study Objectives and Expected Outcomes

The overall objective of the spring 2018 effort is to:

1) Map all large scale Horseshoe Crab spawning areas along the Georgia Coast

2) Collect Red Knot fecal samples at a subset of locations along the Georgia Coast and sequence the prey items

3) Provide information gathered to land managers within the region

The expected outcome of the Red Knot and HSC spawning area assessment is the first map of critical foraging sites fueling the northbound spring migration of Red Knots in Georgia. We expect the DNA barcoding analysis to confirm the connection between HSC eggs and Red Knots. We also expect to highlight sites where human disturbance during spring migration may cause a significant threat to knots.

Statement of Project Activities: 2017-2018

1) *Delineating important HSC spawning areas*– Center for Conservation Biology, Georgia DNR, and Manomet staff visited roughly a dozen sites between 25 April and 7 June to assess HSC spawning densities on the Georgia Coast. CCB staff consulted with local land owners and managers to compile a database of HSC spawning areas. The data is housed in a GIS database within Manomet and CCB, and will be distributed to land managers.

2) *Collecting Red Knot diet samples* – Red Knot fecal samples were collected from foraging and roosting birds, placed in individual vials and fixed in the field for shipment to the College of William & Mary genetics lab for processing.

3) *DNA Barcoding* – In the past decade, mitochondrial DNA sequence analysis, often referred to as DNA bar coding has become a vital tool in the analysis of biodiversity for phylogenetic studies (Hebert, et al. 2003; Kress and Erickson 2012), and has been effectively employed in diet reconstruction studies of avian species (Gerwing, et al. 2016; Joo and Park 2012). To determine whether *Limulus polyphemus* (HSC) eggs and *Donax variabilis* bi-valves are an important part of the diet of the Red Knot (*Calidris canutus*) in coastal Georgia, we analyzed

mitochondrial cytochrome c oxidase subunit I (COI) gene sequences from Red Knot fecal samples.

Similar to the unique pattern of bars in a universal product code (UPC), a DNA barcode is a unique pattern of DNA sequence that identifies each living thing. Barcoding relies on short, highly variably regions of the genome. A region of the mitochondrial gene COI (cytochrome c oxidase subunit I) is used for barcoding animals. DNA is extracted from the target sample, and the barcode portion of the rbcL, COI and ITS gene is amplified by polymerase chain reaction (PCR). The amplified sequence (amplicon) is submitted for sequencing in one or both directions. The sequencing results are then compared to a DNA database housed in GenBank to identify the species in the sample.

Methods

Red Knot Diet Sampling

The fecal samples from foraging Red Knots were collected in coastal Georgia from April to the end of May and immediately placed in individual vials in 1 ml DNAzol Genomic DNA Isolation Reagent (Molecular Research Center, Cat#DN127) and stored at 4°C prior to DNA isolation. As positive controls for polymerase chain reaction (PCR), DNA was also isolated from HSC eggs, *Donax variabilis* (coquina clams), and Zebra Finch muscle tissue.

Samples in DNAzol were vortexed and the liquid portion was collected. After addition of 2 µl Polyacryl Carrier (Molecular Research Center) and 50 µl acid-washed glass beads (MilliporeSigma), samples were pulverized on a BeadRuptor (Omni International) for 1 min at speed setting 5 (5000 rpm). After incubation for 15 min at 55°C, samples were centrifuged for 10 min at 10,000 X g. The clear phase was collected, and DNA was precipitated with 100% ethanol. After washing with 75% ethanol, the DNA pellet was resuspended in sterile H_2O, and samples were placed in Slide-A-Lyzer™ Mini Dialysis devices (ThermoFisher Scientific) and dialyzed against phosphate-buffered saline (PBS), pH 7.4 (Gibco) for 3-4 hrs at 37°C with agitation. Dialyzed DNA samples were quantified by spectrophotometry.

PCR using mitochondrial DNA-specific primers was performed using universal primers and bird-specific primers for the cytochrome oxidase I (COXI) gene (Table 1). Amplification was achieved in 25 µl reactions containing template fecal DNA, 0.5 µM of each primer, and 1X Platinum II Hot-Start PCR MasterMix (Invitrogen). The thermocycling protocol for the universal primers began at 94°C for 1 min; followed by 5 cycles of 94°C for 1 min, 45°C for 1.5 min, 72°C for 1.5 min; followed by 35 cycles of 94°C for 1 min, 50°C

for 1.5 min, 72°C for 1 min; with a final extension of 72°C for 7 min. For the bird-specific primers, the thermocycling protocol began at 95°C for 2 min; followed by 5 cycles of 95°C for 1 min, 46°C for 1 min, 72°C for 30 sec; followed by 45 cycles of 95°C for 1 min, 53°C for 1 min, 72°C for 30 sec; with a final extension of 72°C for 5 min

Table 1. Primer sequences used in this study

Primer name	Primer sequences	Target species	PCR product size	Reference
LCO1490	5′ GGT CAA CAA ATC ATA AAG ATA TTG G 3′	Universal	658 bp	(Hebert et al. 2003)
HCO2198	5′ TAA ACT TCA GGG TGA CCA AAA AAT CA 3′			
K_Bird_F1	5′ CCC CAG ACA TAG CAT TYCC 3′	Birds	226 bp	(Joo and Park 2012)
K_Bird_R1	5′ TTG TGA TAG TGG TGG GGT TTT AT 3′			

PCR products were resolved on 1% agarose gels. Bands were excised and purified using a PCR clean-up kit (IBI Scientific). Sequencing was performed using 1–2 μl sample and the ABI BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems/Life Technologies). Sequencing reactions were purified using Performa® DTR GelFiltration Cartridges (EdgeBio, Gaithersburg, MD), and then were run on an ABI 3500 automated DNA sequencer. Both strands of the products were sequenced using the same primers as for PCR. Sequence chromatograms were visually inspected for ambiguous nucleotides (double peaks). Obtained sequences were aligned using National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) and compared to sequences in GenBank.

Horseshoe Crab Spawning Site Mapping

Center for Conservation Biology staff documented HSC spawning sites from Tybee Island to St Simons Island between 2013 and 2018. CCB consulted with federal, state, and private land managers and biologists to map other known HSC spawning sites on the outer coast. Crab spawning sites were classified as either high use (i.e. likely thousands of spawning crabs), moderate use (likely hundreds), or low use (a few scattered crabs spawning). Crab use of each site was estimated and no mark/recapture data was used to refine these HSC count estimates. Sites were mapped to include the general areas where HSC spawn.

RESULTS

DNA Barcoding of Red Knot Fecal Samples

Red Knot fecal samples generated low and inconsistent yields of DNA, which is typical for fecal samples (Joo and Park 2012). In addition, there was a potent inhibitor of PCR (Schrader, et al. 2012) present in all fecal DNA samples that was not present in samples isolated from HSC eggs, surf clams, or zebra finch muscle tissue. The dialysis step (see Materials and Methods) was critical for improving yield and eliminating the PCR inhibitor. However, only 35% of fecal samples from Tybee Bar and 10% from Little Egg Island generated PCR products that were amenable to DNA sequence analysis.

Of the 49 Red Knot fecal samples collected from Tybee Bar, 33 samples yielded DNA. Of these DNA samples, 17 successfully generated PCR products using the universal primers and were sequenced. One Red Knot fecal sample (TB.F.33) showed *Donax variabilis* (clam) sequences only, though visually all samples collected were dominated by crushed *Donax* shells. The remainder of the samples contained a mixture of sequences from other species, including crustaceans, flies, mosquitos, beetles, algae, diatoms, and bacteria.

Of the 30 Red Knot fecal samples collected at Little Egg Island, 19 samples yielded DNA. Of those, 3 resulted in sequenced PCR products. Two Red Knot (LEI.F.27 and LEI.F.29) samples showed *Limulus polyphemus* (HSC) sequences only, though visually all samples collected on Little Egg Island were comprised primarily of partially digested HSC eggs. The remaining sample contained a mixture of sequences from other species, including mosquitos and diatoms. Finally, PCR and sequencing with bird-specific COXI primers was performed for several samples; positive results confirmed that fecal samples were from Red Knots.

Horseshoe Crab Spawning Site Mapping

Sites were visited multiple times throughout several field seasons between 2013 and 2018 (see Figure 1). There are dozens of saltmarsh sites where HSC are spawning annually in Georgia, and the vast majority of these sites are either inaccessible to shorebirds due to vegetation density or spawning density is not high enough to draw shorebirds to the site. The focus of this study is to identify spawning sites where Red Knots access the food resource, primarily on the outer coastal barrier islands and sandbars.

Site Summaries

Tybee Bar – Tybee Bar is located on the seaward side of Little Tybee Island (Figure 2). Tybee bar was a low use HSC site in 2015, and no spawning at the site was observed in either 2016 or 2018. Few Red Knots or other shorebirds were observed utilizing this resource in 2015. Tybee bar is utilized by large numbers of Red Knot centered on mid-falling to mid-rising tides (Smith et al 2017, F. Smith pers. obs. 2018) where birds forage on abundant *Donax* clams along the tidal edge. The bar is completely submerged on each high tide event.

Little Tybee Island/Beach Hammock –Little Tybee Island/Beach Hammock (Figure 2) was a high use spawning site in both 2015 and 2016. During the 2017 and 2018 seasons, dramatic changes in the structure of the sand bar located near the southern tip of the island resulted in low use of the location for HSC spawning. This site had many thousands of Red Knots utilizing the resource in 2015 and 2016 (Smith et al 2017) and only low hundreds of knots seen there on two occasions in spring of 2017 and 2018. The middle inlet on Little Tybee showed some evidence of low numbers of HSC spawning during the 2015 season but was not visited in 2016 or 2018 due to accessibility issues. Feral hog depredation of HSC eggs on Beach Hammock was observed during the 2015 spring season.

Ogeechee Bar/Raccoon Key Complex – The Ogeechee Bar/Raccoon Key sites (Figure 3) were high use HSC spawning sites in each year of the study (2013, 2015-2018). Stable concentrations of large numbers of knots (>1000, from Smith et al. 2017) were observed at Ogeechee Bar each spring just prior to departure of the birds from the Southeast Coast (2nd to 3rd week of May), suggesting that the Ogeechee Bar is one of the most important stopover sites in the region. Egg Island was the only marsh site between 2013 and 2018 where Red Knots were observed foraging on HSC eggs. Spawning occurred on Egg Island in the short-form of *Spartina* grass and on shell rake. The site near the Ossabaw Island dock is a low to moderate use site, and no shorebirds were observed feeding on HSC eggs at this site. Feral hogs were observed feeding on spawning crabs at the Ossabaw dock location in multiple years in spite of an intense hog population management program on the island.

St Catherines Bar and Island – The St Catherines Island sites (Figure 4) were visited during the 2016 field season, and we documented abundant HSC spawning on the north and south ends of the island and at McQueen Inlet. Feral hogs were observed feeding on spawning crabs on the north end of the island in

2015 and 2016, and follow up with island managers to determine effectiveness of the management initiative is a priority. The north and south end of the island was visited during the 2018 spring season and high use spawning was observed in both locations.

Cabretta/Blackbeard/Sapelo Islands –A tidal pond site located on the seaside of Cabretta Island (Figure 5) provided prime high use HSC spawning habitat in 2013, but a subsequent storm closed the tidal inlet to the spawning site. No use of Cabretta Island was documented in the 2015, 2016, or 2017 seasons, and very low use was documented on the south end of Blackbeard Island during those seasons. Feral hog depredation of gravid female HSCs was a significant issue on the north end of the island in 2015 and 2016, though management in recent years may have that problem under control.

Altamaha River to Gould's Inlet – These sites, from north to south, include Little Egg Island and Little Egg Bar, Little St Simons Island, Pelican Spit, and Gould's Inlet (Figure 6). High spawning use was documented on Little Egg Island in multiple years and on Pelican Spit in 2013 and 2015. Low HSC spawning use was observed on the north end of Little St Simons Island, and moderate use was documented on the Rainbow Beach inlet on the south end of the island in 2013 and from 2015-2018. Gould's Inlet was a low use HSC spawning site during the 2015 season, with small numbers spawning near the highest ridge of the bar. Little to no spawning was observed at Gould's in 2013 or in 2016-2018.

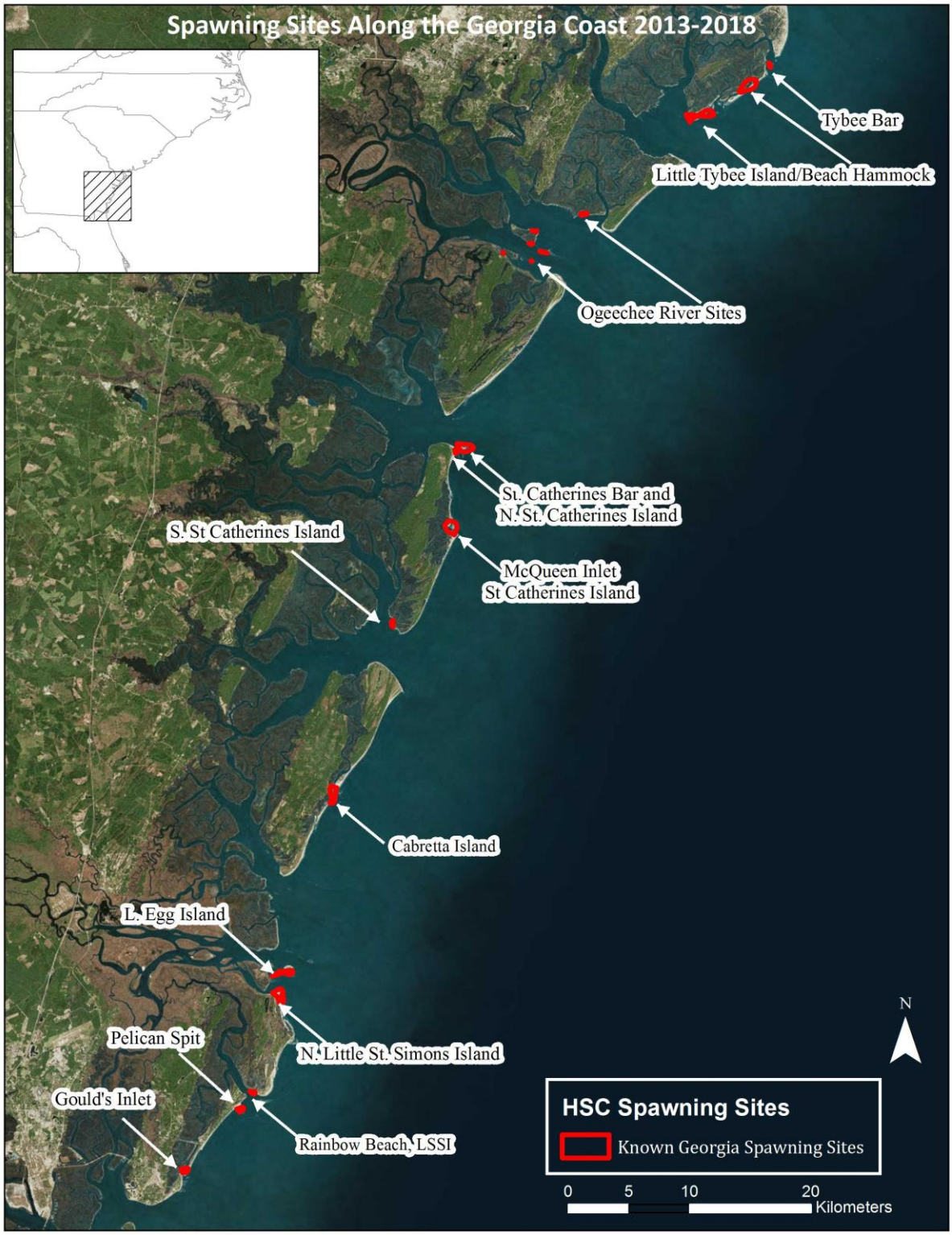

Figure 1. Horseshoe Crab spawning sites from 2013-2018 seasons along the Georgia coast.

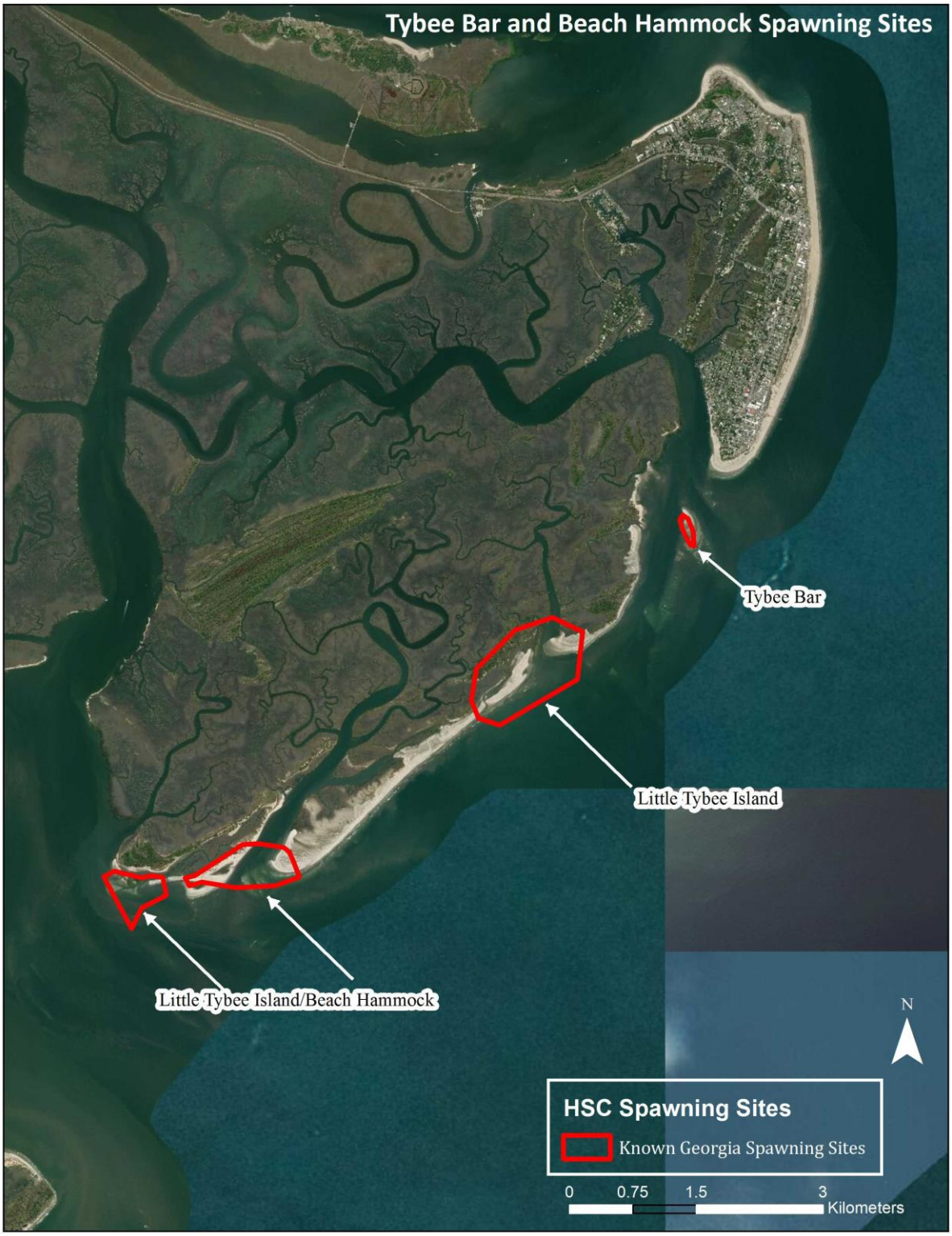

Figure 2. Horseshoe Crab spawning sites from 2013-2018 seasons at Tybee Bar and Little Tybee Island/Beach Hammock.

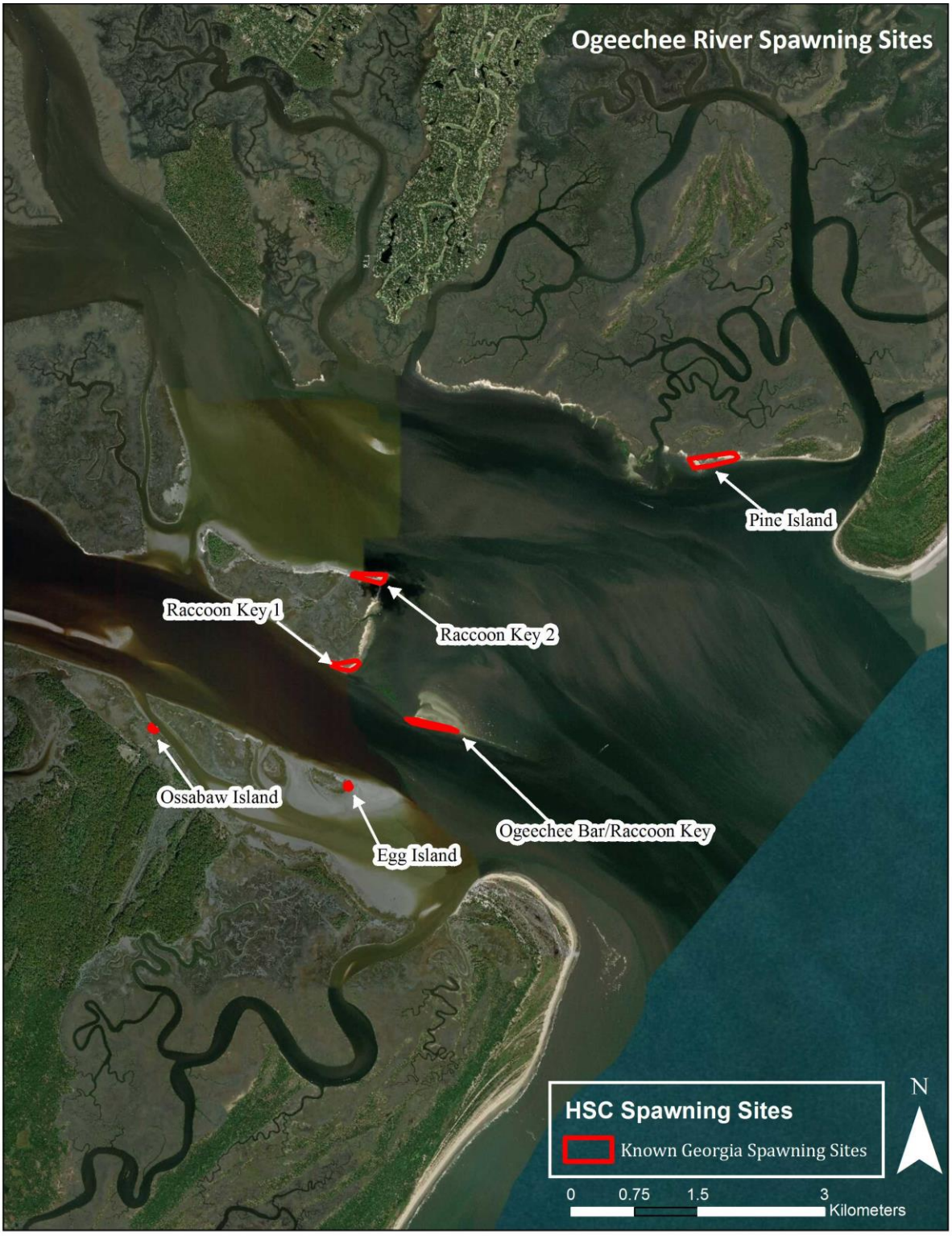

Figure 3. Horseshoe Crab spawning sites from 2013-2018 seasons in the Ogeechee River mouth.

Figure 4. Horseshoe Crab spawning sites from 2013-2018 seasons on St Catherines Island.

Figure 5. Horseshoe Crab spawning sites from 2013-2018 seasons on Sapelo, Cabretta, and Blackbeard Islands.

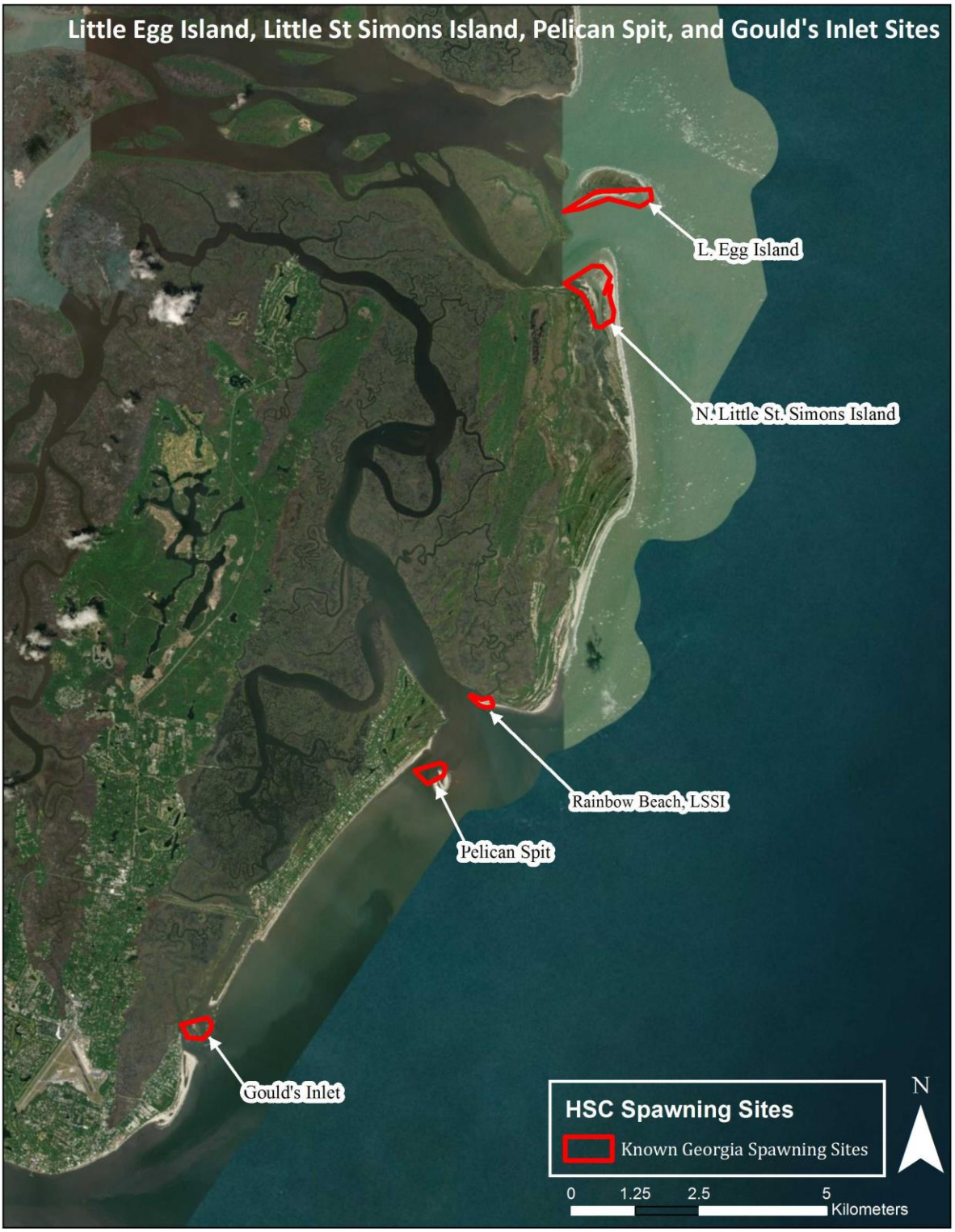

Figure 6. Horseshoe Crab spawning sites from 2013-2018 seasons between Little Egg Island and Gould's Inlet.

DISCUSSION and PROJECT OUTCOMES

Previous research along the Georgia coast has shown the importance of the region for migratory Red Knots. The superpopulation moving through during fall migration has exceeded 23,000 birds (Lyons et al 2017) and ranged between 8,000 and 14,000 during a three year spring migration study (Smith et al 2017). The most recent population estimate for the *rufa* subspecies is 42,000 individuals (Andres et al. 2012). Georgia Red Knot research has shown unequivocally that a high proportion of the population utilizes the Georgia coast each spring, and in some years over half of the population is using the Georgia coast in fall migration. Recent work conducted in South Carolina has shown that many Red Knots are staging in the Southeast and flying directly to breeding grounds (Burger 2012, SC DNR unpublished data) suggesting that the region is far more important to the long-term recovery of the species than previously thought.

Red Knots stratify foraging based on tidal stage while in Georgia. Horseshoe Crabs spawn on higher elevation areas of islands and bars along the coast. We documented 11 high use crab spawning sites between 2013 and 2018, and at least 5 had high use spawning numbers during the 2018 season (Ogeechee Bar and Raccoon Key each with a site, north and south St Catherines Island, and Little Egg Island). Spawning is initiated based on water temperature and moon cycle, and a large spawning event was observed on April 25th, with spawning observed on full and new moon tides through the spring migration season into late May. Red Knots are the best indicator of spawning magnitude along the coast (Karpanty et al. 2006) and high concentrations of knots are observed in all coastal high use spawning sites between April and the 3rd week of May. HSC eggs provide an easily digestible food resource (Tsipoura and Burger 1999, Robinson et al. 2003, Atkinson et al. 2007, Mizrahi and Peters 2009) and Red Knots have been shown to utilize HSC eggs elsewhere in the Southeast USA (Takahashi 2016, Smith et al. 2017). Many of the Georgia HSC spawning sites had variable levels of spawning activity between years; for instance Little Tybee Island/Beach Hammock had the highest number of crabs spawning along the coast in 2015, but only a low use spawning site was found on the island during the 2018 season. Cabretta Island had high numbers of crabs spawning in 2013 but was a low to no use site in 2015, 2016, and 2018.

Horseshoe Crabs are harvested for conch and eel bait, and for the medical industry. There is no current large-scale harvest of HSCs in Georgia, but adjacent South Carolina has seen a significant (possibly unsustainable) increase in harvest (Takahashi 2016, F. Sanders pers. comm.). Crab harvest management remains a contentious issue in the Delaware Bay Red Knot stopover site (Odell et al. 2005). Feral hog depredation of gravid female Horseshoe Crabs and of eggs was observed at several sites, including Little Tybee Island/Beach Hammock, St Catherines Island, and Ossabaw Island. Ossabaw and St Catherines both have ongoing hog management programs initiated to reduce sea turtle nest depredation. It is unknown what impact of feral hog depredation has on the HSC population. On one section of beach on north St Catherines Island there were hundreds of depredated female crab carcasses during the 2015 and 2016 seasons. The topic should be addressed and monitored moving forward.

Knots focus their foraging on high use HSC spawning sites at or near high tide (a 1 to 3 hour window), and when tidal conditions are right they form short-lived mixed-species feeding frenzies around HSC nests. HSC eggs are inaccessible during mid-falling to mid-rising tides, and Red Knots forage during these tides primarily on *Donax* clams. Large numbers (>250) of Red Knots were observed feeding on *Donax* on Tybee Bar, Ogeechee Bar, Ossabaw Island, St. Catherines Bar, Cabretta/Blackbeard Bar, Wolf Bar, Little St. Simons Island, and Gould's Inlet (Smith et al 2017). Between 2013 and 2018, Low tide foraging sites (Tybee Bar, Ossabaw Island, Cabretta/Blackbeard Bar, St Catherines Bar, and Gould's Inlet showed less annual variability in Red Knot use, suggesting a relatively stable population of *Donax* clams at those sites in contrast to the cyclical *Mulinia lateralis* population of the Georgia coast (Lyons et al 2017) or the highly dynamic and variable HSC spawning sites.

In recent years, DNA barcoding has been used to investigate diet (Pompanon et al. 2012). The technique is particularly useful for species living in remote environments or where observations are difficult to obtain (Valentini et al. 2016) such as fish (Riemann et al. 2011, Cote et al. 2013) and large herbivores (Kartzinel et al. 2015). Use of the technique to evaluate diet from feces of bats (Zeale et al. 2011, Alberdi et al. 2012) and birds (Deagle et al. 2010) is particularly attractive and may represent a breakthrough for a large number of species where collecting diet information has been problematic. Shorebirds that forage on small prey represent good candidates for this technique. We encountered limitations of DNA analysis in the processing of the Red Knot fecal samples collected during the 2018 spring season. Visually, all samples collected on Tybee Bar were dominated by crushed *Donax* shells, and all samples collected on Little Egg Island were comprised of partially digested HSC eggs (F. Smith pers. obs.). The degree of DNA degradation in the main prey items (*Donax* and HSC eggs) appears to be high and PCR likely amplified the more intact DNA (non-digested) resulting in erroneous diet results. The amplified DNA items found in the majority of samples do not appear to be an important part of the diet.

The relationship between Red Knots and their prey base in the Southeastern US is poorly understood. Recent studies have shed light on some aspects of the boom/bust nature of both prey and knot densities (Harrington et al. 2007, Lyons et al. 2017), and distribution of Red Knots in relation to high use HSC spawning sites and sites with high densities of *Donax* (Smith et al. 2017). This study showed the relationship between tide stage and prey base which was previously unreported. One significant gap in knowledge regarding prey species is a complete lack of study quantifying prey densities and distribution. A recent collapse of the *Donax* population in a nearby location (Fernandina Beach, FL, P. Leary pers. comm.) has resulted in a simultaneous abandonment of the area by numbers of Red Knots. The factors driving the fluctuations of the *Mulinia* population are poorly understood, as are the factors driving the density and distribution of *Donax* clams and Horseshoe Crab spawning sites. Dedicated work to determine these factors should be considered a high priority to better understand the dynamic relationship between Red Knots and their prey along the Southeastern coast. Human disturbance at several sites with high numbers of foraging Red Knots remains an issue on the coast. Varying levels of disturbance were observed at Tybee Bar, Little Tybee Island/Beach Hammock, Ogeechee Bar/Raccoon

Key, Cabretta/S. Blackbeard bar, and Pelican Spit. Though each of these sites varies in importance between years, overall the region is critically important to a high percentage of the overall Red Knot population. At the very least, a baseline of prey data is necessary to inform future management decisions where human disturbance is an issue. Development and implementation of standardized protocols for sampling crab spawning and egg densities and bivalve densities should be a high research priority in the region.

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